EXPRESS MAIL LABEL NO. EL874429111US
DATE OF DEPOSIT: December 17, 2001

JC07 Rec'd PCT/PTO 17 DEC 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER	
5585-61534	
U S APPLICATION NO (If known, see 37 C F R § 1 5)	
Not Yet Assigned	10/018608

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/02216	19 June 2000	18 June 1999

TITLE OF INVENTION
BIOLOGICALLY ACTIVE MATERIALS

APPLICANT(S) FOR DO/EO/US
Ruth Duncan, Dale Hreczuk-Hirst and Lisa German

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U S C § 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U S C § 371.
3. ☐ This is an express request to begin national examination procedures (35 U S C § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U S C § 371(b) and PCT Articles 22 and 39(1)
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. ☒ A copy of the International Application as filed (35 U S C § 371(c)(2))
   a ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b ☐ has been transmitted by the International Bureau
   c ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U S C § 371(c)(2))
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U S C § 371(c)(3))
   a ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b ☐ have been transmitted by the International Bureau
   c ☐ have not been made, however, the time limit for making such amendments has NOT expired.
   d ☒ have not been made and will not be made
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C § 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U S C § 371(c)(4)) (Unsigned)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S C § 371(c)(5))

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C F R §§ 1 97 and 1.98
12. ☐ An assignment document for recording A separate cover sheet in compliance with 37 C F.R §§ 3 28 and 3 31 and the Recordal fee of $40 00 is included
13. ☒ A **FIRST** preliminary amendment
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
14. ☐ A substitute specification
15. ☐ A change of power of attorney and/or address letter
16. ☒ Other items or information
    ☒ Written Opinion
    ☒ Preliminary Examination Report
    ☒ International Search Report.
    ☒ Copies of References Cited

24197

WDN/SAS/GLB:clm 12/17/01 5585-61534 85991.doc

EXPRESS MAIL LABEL NO. EL874429111US
DATE OF DEPOSIT: December 17, 2001

U.S. APPLICATION NO (If known, see 37 C.F.R. § 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
Not Yet Assigned 10/018608	PCT/GB00/02216	5585-61534

	CALCULATIONS (PTO USE ONLY)
17. ☒ The following fees are submitted.	
BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):	
Neither International Preliminary Examination fee (37 C.F.R. § 1 482) nor International Search fee (37 C.F.R. § 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1,040.00**	
International Preliminary Examination fee (37 C.F.R. § 1 482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
International Preliminary Examination fee (37 C.F.R. § 1.482) not paid to USPTO but International Search fee (37 C F R § 1.445(a)(2)) paid to USPTO as an International Searching Authority **$740.00**	
International Preliminary Examination fee paid to USPTO (37 C.F.R. § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
International Preliminary Examination fee paid to USPTO (37 C F.R. § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C F.R. § 1 492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	21 - 20 =	1	x $18.00	$ 18.00
Independent Claims	3 - 3 =	0	x $84.00	$ 0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$ 0.00
TOTAL OF ABOVE CALCULATIONS =				$ 908.00
☐ Reduction of 1/2 for filing by small entity Small entity status is claimed for this application.				$ 0.00
SUBTOTAL =				$ 908.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C F R §§ 1 492(f)). +				$ 0.00
TOTAL NATIONAL FEE =				$ 908.00
Fee for recording the enclosed assignment (37 C.F.R § 1 21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C F R. §§ 3.28, 3 31) **$40.00** per property +				$ 0.00
TOTAL FEES ENCLOSED =				$ 908.00
			REFUND →	$
			CHARGE →	$

a. ☒ A check in the amount of $908 00 to cover the above fees is enclosed

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No 02-4550. A duplicate copy of this sheet is enclosed

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 S W Salmon Street
Portland, OR 97204-2988

William D Noonan
SIGNATURE
William D Noonan, M.D.
NAME

30,878
REGISTRATION NUMBER

cc: Docketing

WDN/SAS/GLB:clm 12/17/01 85509 doc
Attorney Reference Number 5585-61534

Express Mail Label No.: EL874429111US
Date of Deposit: December 17, 2001
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Duncan *et al.*

Application No. Not Yet Assigned

Filed: Herewith

For: BIOLOGICALLY ACTIVE MATERIALS

Examiner: Not Yet Assigned

Date: December 17, 2001

Art Unit: Not Yet Assigned

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on December 17, 2001 as U S Express Mail in an envelope addressed to: BOX PCT, Commissioner for Patents, Washington, D.C. 20231.

William D Noonan

William D Noonan, M.D., J.D.
Attorney for Applicant

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination of the above-referenced application, please amend the application as follows:

In the Specification:

On page 1, line 2, please insert the following:

PRIORITY CLAIM

This is a U.S. National Stage § 371 of PCT/GB00/02216, filed June 19, 2000, which was published in English under PCT Article 21(2), which claims the benefit of U.K. Application GB9914187.1, filed June 18, 1999, and U.K. Application GB9930252.3, filed December 22, 1999.

In the Claims:

Please cancel pending claims 14 and 15 without prejudice. Please amend pending claims 2-13 and 16-23 as follows:

1. (Reiterated) A polymer drug conjugate comprising:
at least one anti-cancer agent; and

a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterised in that the stability of the polymer drug conjugate is enhanced.

2. (Amended) The polymer drug conjugate according to Claim 1, wherein said dextrin is succinoylated to at least 30mol%.

3. (Amended) The polymer drug conjugate according to Claim 2, wherein said dextrin is succinoylated from 30% to 40mol%.

4. (Amended) The polymer drug conjugate according to Claim 3, wherein said dextrin is succinoylated from 32% to 36mol%.

5. (Amended) The polymer drug conjugate according to Claim 4, wherein said dextrin is succinoylated to about 34mol%.

6. (Amended) The polymer drug conjugate according to Claim 1, wherein a percentage of α-1-6 linkages in the dextrin is less than 10%.

7. (Amended) The polymer drug conjugate according to Claim 6, wherein the percentage of α-1-6 linkages in the dextrin is less than 5%.

8. (Amended) The polymer drug conjugate according to Claim 1, wherein a molecular weight of the dextrin is in an average molecular weight range 1000-200000.

9. (Amended) The polymer drug conjugate according to Claim 8, wherein a molecular weight of the dextrin is in an average molecular weight range 2000-55000.

10. (Amended) The polymer drug conjugate according to Claim 1, wherein the dextrin contains more than 15% of polymers of DP greater than 12.

11. (Amended) The polymer drug conjugate according to Claim 10, wherein the dextrin contains more than 50% of polymers of DP greater than 12.

12. (Amended) The polymer drug conjugate according to Claim 1, wherein said anti cancer agent is selected from the group consisting of: cyclophosphamide; melphalan; carmusline; methotrexate, 5-fluorouracil; cytarabine; mercaptopurine; anthracyclines; daunorubicin; doxorubicin; epirubicin, vinca alkaloids; vinblastin, vincristine; dactinomycin; mitomycin C; taxol; L-asparaginase; G-CSF; cisplatin; and carboplatin.

13. (Amended) A pharmaceutical composition, comprising the polymer drug conjugate according to Claim 1 and a pharmaceutically acceptable diluent, excipient or carrier.

14. Please cancel claim 14.

15. Please cancel claim 15.

16. (Amended) A polymer drug conjugate comprising:
at least one biologically active agent; and
a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterized in that the stability of the polymer drug conjugate is enhanced.

17. (Amended) The polymer conjugate according to Claim 16, wherein said agent is an imaging agent.

18. (Amended) The polymer conjugate according to Claim 17, wherein the imaging agent is tyrosinamide.

19. (Amended) The polymer conjugate according to Claim 16, wherein said agent is a diagnostic agent.

20. (Amended) The polymer conjugate according to Claim 16, wherein said agent is a targeting agent.

21. (Amended) The polymer conjugate according to Claim 20, wherein the targeting agent is biotin.

22. (Amended) A method for treating a disease or disorder in an animal subject, comprising:

administering to the animal a pharmaceutically effective amount of the polymer drug conjugate according to Claim 1, thereby treating the disease or disorder in the subject.

23. (Amended) The method according to Claim 22, wherein said animal is human.

REMARKS

By this amendment the specification has been changed to reflect prior related applications. No new matter is added by this amendment.

Claims 15 and 16 are cancelled herein without prejudice. Claims 2-13 and 16-23 are amended to correct form or to remove multiple dependencies in order to reduce the filing fee.

No new matter has been added by this amendment. Examination of the subject application is respectfully requested.

CONCLUSION

If any minor matters need to be addressed, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By: William D Noonan
William D. Noonan, M.D., J.D.
Registration No. 30,878

One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, Oregon 97204
Telephone: (503) 226-7391
Facsimile: (503) 228-9446

Marked-up Version of Amended Claims and Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the Specification:

Page 1, line 2, please insert the following:

--PRIORITY CLAIM

This is a U.S. National Stage § 371 of PCT/GB00/02216, filed June 19, 2000, which was published in English under PCT Article 21(2), which claims the benefit of U.K. Application GB9914187.1, filed June 18, 1999, and U.K. Application GB9930252.3, filed December 22, 1999.--

In the Claims:

Please amend the claims as follows:

1. (Reiterated) A polymer drug conjugate comprising:
at least one anti-cancer agent; and
a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterised in that the stability of the polymer drug conjugate is enhanced.

2. (Amended) [A] The polymer drug conjugate according to Claim 1, wherein said dextrin is succinoylated to at least 30mol%.

3. (Amended) [A] The polymer drug conjugate according to Claim 2, wherein said dextrin is succinoylated from 30% to 40mol%.

4. (Amended) [A] The polymer drug conjugate according to Claim 3, wherein said dextrin is succinoylated from 32% to 36mol%.

5. (Amended) [A] The polymer drug conjugate according to Claim 4, wherein said dextrin is succinoylated to about 34mol%.

6. (Amended) [A] The polymer drug conjugate according to [any of Claims] Claim 1[-5], wherein [the] a percentage of α-1-6 linkages in the dextrin is less than 10%.

7. (Amended) [A] The polymer drug conjugate according to Claim 6, wherein the percentage of α-1-6 linkages in the dextrin is less than 5%.

8. (Amended) [A] The polymer drug conjugate according to [any of Claims] Claim 1,[-7] wherein [the] a molecular weight of the dextrin is in [the] an average molecular weight range 1000-200000.

9. (Amended) [A] The polymer drug conjugate according to Claim 8, wherein [the] a molecular weight of the dextrin is in [the] an average molecular weight range 2000-55000.

10. (Amended) [A] The polymer drug conjugate according to [any of Claims] Claim 1[-9], wherein the dextrin contains more than 15% of polymers of DP greater than 12.

11. (Amended) [A] The polymer drug conjugate according to Claim 10, wherein the dextrin contains more than 50% of polymers of DP greater than 12.

12. (Amended) [A] The polymer drug conjugate according to [any of Claims] Claim 1[-13], wherein said anti cancer agent is selected from the group consisting of: cyclophosphamide; melphalan; carmusline; methotrexate, 5-fluorouracil; cytarabine; mercaptopurine; anthracyclines; daunorubicin; doxorubicin; epirubicin, vinca alkaloids; vinblastin, vincristine; dactinomycin; mitomycin C; taxol; L-asparaginase; G-CSF; cisplatin; and carboplatin.

13. (Amended) A pharmaceutical composition, comprising [a] the polymer drug conjugate according to [any of Claims] Claim 1[-12] and a pharmaceutically acceptable diluent, excipient or carrier.

14. Please cancel claim 14.

15. Please cancel claim 15.

16. (Amended) A polymer drug conjugate comprising:

[i)] at least one biologically active agent; and

[ii)] a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterized in that the stability of the polymer drug conjugate is enhanced.

17. (Amended) [A] The polymer conjugate according to Claim 16, wherein said agent is an imaging agent.

18. (Amended) [A] The polymer conjugate according to Claim 17, wherein the imaging agent is tyrosinamide.

19. (Amended) [A] The polymer conjugate according to Claim 16, wherein said agent is a diagnostic agent.

20. (Amended) [A] The polymer conjugate according to Claim 16, wherein said agent is a targeting agent.

21. (Amended) [A] The polymer conjugate according to Claim 20 wherein the targeting agent is biotin.

22. (Amended) A method [of treatment of] for treating a disease or disorder in an animal subject, [the method including the administration of] comprising:

administering to the animal a pharmaceutically effective amount of the polymer drug conjugate according to [any of Claims] Claim 1[-12], thereby treating the disease or disorder in the subject.

23. (Amended) [A] The method [of treatment] according to Claim 22, wherein said animal is human.

BIOLOGICALLY ACTIVE MATERIALS

Field of Invention

This invention relates to biologically active materials and, in particular, to materials which comprise a biodegradable polymer linked to a biologically active agent. The invention is concerned with materials known as polymer-drug conjugates which typically contain a therapeutic agent for instance, a bioactive cytotoxic drug, linked to a polymer back-bone. The linkage between the polymer and the drug is typically by covalent bonding. However, the invention is applicable to other polymer conjugates including those where the biologically active agent is an imaging agent, such as tyrosinamide, a diagnostic agent, or a targeting agent such as biotin.

Reference will be made hereinbelow to polymer-drug conjugates in which the drugs are anticancer agents. However, the present invention has application in connection with other drugs and/or bioactive agents.

Background of the Invention

In designing a polymer-drug conjugate, the aim is to deliver a drug effectively to a therapeutic site such as a tumour. It is known, for instance, that polymer-drugs given intravenously can accumulate selectively in solid tumour tissue by the EPR effect.

The most commonly used anticancer agents are low molecular weight compounds which readily gain access to cells by rapid passage across the cell membrane. After intravenous (IV) administration, a large percentage of the injected dose leaves the circulation within a few minutes, resulting in a ubiquitous body distribution of drug and little selective concentration in tumour tissue. By creating a macromolecular polymer-anticancer drug conjugate, there is provided an opportunity to improve tumour specific targeting, to minimise drug entry into sites of toxicity, to control precisely the rate of drug liberation at the target site (giving opportunities for long-

term controlled release) and to deliver the active principal intracellularly, thereby providing a means to overcome p-glycoprotein related multidrug resistance.

Numerous polymers have been proposed for synthesis of polymer-drug conjugates including polyaminoacids, polysaccharides such as dextran, and synthetic polymers such as N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer. However, these polymers have limitations. For example, a dextran-doxorubicin conjugate has been tested clinically and been found to be much more toxic than the parent drug. Furthermore the HPMA copolymers which have been clinically tested have the disadvantage of being non-biodegradable in the main chain.

WO-A-98/56424 discloses a polymer-drug conjugate in which the polymer is the polysaccharide dextrin. Such a polymer-drug conjugate may be prepared in various ways. One method involves succinoylating dextrin and reacting the succinoylated dextrin with the drug or a reactive derivative thereof.

WO-A-98/56424 includes an example in which the extent of succinoylation of dextrin varies from 2.26 to 6.64 Mol%. In a further example the drug doxorubicin is conjugated to succinoylated dextrins in which the extent of succinoylation varies from 0.5 to 14.9 Mol%.

WO-A-98/56424 also includes examples showing the rate of degradation of dextrin both in the absence and in the presence of appropriate enzymes and also in rat plasma.

For at least certain applications the rate of degradation of dextrin in a dextrin-drug conjugate is an important consideration. For instance, it may be desirable to have a relatively slow rate of degradation in some applications while in other applications a faster rate of degradation is either acceptable or indeed even preferred.

Statement of Invention

It has now been surprisingly discovered that the rate of dextrin degradation is highly dependent on the degree of dextrin backbone substitution. As a result, it is possible to tailor the dextrin by appropriate substitution of its backbone in order to achieve a desired rate of degradation.

According to a first aspect of the invention there is provided a polymer drug conjugate comprising:

i) at least one anti-cancer drug; and

ii) a dextrin polymer

characterised in that said dextrin polymer is modified by the addition of pendent groups so that the stability of the polymer drug conjugate is enhanced.

The term "dextrin" means a glucose polymer which is produced by the hydrolysis of starch and which consists of glucose units linked together by means mainly of alpha-1,4 linkages. Typically dextrins are produced by the hydrolysis of starch obtained from various natural products such as wheat, rice, maize and tapioca. In addition to alpha-1,4 linkages, there may be a proportion of alpha-1,6 linkages in a particular dextrin, the amount depending on the starch starting material. Since the rate of biodegradability of alpha-1,6 linkages is typically less than that for alpha-1,4 linkages, for many applications it is preferred that the percentage of alpha-1,6 linkages is less than 10% and more preferably less than 5%.

Any dextrin is a mixture of polyglucose molecules of different chain lengths. As a result, no single number can adequately characterise the molecular weight of such a polymer. Accordingly various averages are used, the most common being the weight average molecular weight (Mw) and the number average molecular weight (Mn). Mw is particularly sensitive to changes in the high molecular weight content of a polymer whilst Mn is largely influenced by changes in the low molecular weight of the polymer.

It is preferred that the Mw of the dextrin is in the range from 1,000 to 200,000, more preferably from 2,000 to 55,000.

The term 'degree of polymerisation' (DP) can also be used in connection with polymer mixtures. For a single polymer molecule, DP means the number of polymer units. For a mixture of molecules of different DP's, weight average DP and number average DP correspond to Mw and Mn. In addition DP can also be used to characterise a polymer by referring to the polymer mixture having a certain percentage of polymers of DP greater than a particular number or less than a particular number.

It is preferred that, in the dextrin-drug conjugate of the present invention, the dextrin contains more than 15 % of polymers of DP greater than 12 and, more preferably, more than 50% of polymers of DP greater than 12.

Modifications to dextrin may be negatively charged groups, neutral groups or positively charged groups, (eg quaternary ammonium groups).

In a further preferred embodiment of the invention said dextrin modification is succinoylation.

In a yet further preferred embodiment of the invention said dextrin succinoylation is greater than 20 mol %. Preferably said dextrin succinoylation is at least 30mol%. More prefereably still said succinoylation is from 30% to 40%.

More preferably still said succinoylation is 30mol%; 31mol%; 32mol%; 33mol%; 34mol%; 35mol%; 36mol%; 37mol%; 38mol%; 39mol%; 40mol%. Ideally said succinoylation is 34mol%.

In a yet further preferred embodiment of the invention said succinoylated dextrin comprises an anti-cancer agent selected from: cyclophosphamide; melphalan; carmusline; methotrexate, 5-fluorouracil; cytarabine; mercaptopurine; anthracyclines; daunorubicin; doxorubicin; epirubicin; vinca alkaloids; vinblastin; vincristine; dactinomycin; mitomycin C; taxol; L-asparaginase; G-CSF; cisplatin; carboplatin.

More preferably still said anti-cancer agent is doxorubicin.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising a polymer drug conjugate according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said composition comprises a diluent, carrier or excipient.

In a further preferred embodiment of the invention said polymer drug conjugate is for use in the manufacture of a medicament for the treatment of cancer.

According to a further aspect of the invention there is provided a method to treat an animal, ideally a human being, suffering from cancer by administration of the polymer drug conjugate according to the invention.

It has been found that, in the case of substitution of the dextrin backbone by succinoylation, relatively rapid degradation takes place at a degree of succinoylation of up to about 15%. By contrast a degree of succinoylation above 30% very markedly reduces the rate of degradation.

The present invention provides a dextrin-drug conjugate in which the degree of substitution of the dextrin chain is greater than 15%, more preferably greater than 20% and most preferably greater than 30%.

The drug of the dextrin-drug conjugate may be loaded on the polymer via a linking group, such as succinoyl, in which case it may be attached to some or all of the linking groups. Alternatively the drug may be directly loaded onto the dextrin backbone in which case the drug itself acts as the substituting group. As a further possibility the drug may be loaded partly via a substituting group and partly directly onto the dextrin backbone.

An embodiment of the invention will now be described by example only and with reference to the following tables and figures;

Table 1 represents the characteristics of different batches of succinoylated dextrin doxorubicin conjugates;

Table 2 shows the anticancer activity of succinoylated dextrin doxorubicin conjugates;

Figure 1 is a graphical representation of the degradation of dextrin, succinoylated dextrin and a succinoylated dextrin doxorubicin conjugate (5% succinoylation, 6% doxorubicin);

Figure 2 is a graphical representation of the degradation of hyper-succinoylated dextrin doxorubicin(34% succinoylation) conjugate with time;

Figure 3 is a graphical representation of the preferential accumulation of succinoylated dextrin doxorubicin conjugate compared to an unconjugated control;

Figure 4 illustrates the effect of the degree of dextrin succinoylation on biodistribution of ^{125}I-labelled Dextrin at 34mol% after i.v. administration;

Figure 5 illustrates a comparison of the 1 and 34 mol% modified ^{125}I-labelled dextrin at 5 min post i.v administration;

Figure 6 illustrates a comparison of the 1 and 34 mol% modified ^{125}I-labelled dextrin at 1hr post i.v administration; and

Figure 7 represents the presence of ^{125}I –labelled dextrin in the peritoneal wash after i.p. administration at 1hr

Detailed description of the invention

Example 1

Dextrin (Mw 51,000 Da) was succinoylated using a modification of the method described by Bruneel *et al* (Polymer, 35 (12),(1994), 2656-2658). Doxorubicin was then conjugated directly via an amide bond, conjugated via an N-*cis*-aconityl spacer or conjugated via a glycyl-N-*cis*-aconityl spacer.

Polymer degradation (unmodified dextrin, succinoylated dextrin (5, 15 mol %) and conjugate) was measured in the presence of amylase or lysosomal enzymes to monitor either changes in polymer molecular weight (GPC) or doxorubicin release (HLPC).

The dextrin-doxorubin conjugates had a doxorubicin loading of 6-12 wt% dependent on the reaction conditions used and the degree of succinoylation of the dextrin intermediate. Table 1 shows the characteristics of several batches of dextrin-succ-doxorubicin.

Table 1 Characteristics of batches of dextrin-succ-doxorubicin

Batch No	Dox (wt%)	Free Dox (% total Dox)
1	11.7	0.8
2	11.9	2.0
3	8.7	1.2
4	8.4	0.1

After a 180 min incubation with amylase, unmodified dextrin is almost completely degraded to low molecular products, whilst the succinoylated dextrin (5 and 15 mol %) and dextrin-succ-doxorubicin show a biphasic pattern of degradation giving rise to fragments of Mw 4,000, 9,500 and 6,400 Da respectively. Unmodified dextrin had a $t_{½}$ (time for mass to reach half of its original) of 20 min, succinoylated dextrin and dextrin-succ-doxorubicin a $t_{½}$ of approximately 15 min.

Example 2

In this example the degradation of dextrins of different degrees of modification was compared. The results are shown in Figure 1. It will be seen that native dextrin is rapidly degraded as are also dextrin with 5% succinoylation (whether with or without 6% Dox) and dextrin with 15% succinoylation. However, if dextrin is 34% succinoylated the degree of degradation is markedly less, there being zero% reduction of the peak mass of primary peak after 60 minutes and only 20% reduction after 180 minutes. In addition, Figure 2 shows that 34% succinoylated dextrin doxorubicin conjugate is similarly stable over an extended time course when compared to unconjugated or low level succinoylated (5%) controls.

Example 3

In this example increased uptake of 34% succinoylated dextrin-doxorubicin by tumour cells is shown. Male C57 were injected with 10^6 B16F10 murine melanoma cells subcutaneously with either doxorubicin hydrochloride or dextrin- succinoyl-doxorubicin (34 mol % succinoylation, 11.8% doxorubicin) at 5mg/kg doxorubicin equivalence into the intrapertinoneal cavity (i.p.).

The mice were then culled after 2, 5, and 30 mins and after 1, 2, 5, 24, and 48 hours. Tumours were removed and weighed. The tumour was then homogenised and doxorubicin extracted and quantified by HLPC for total doxorubicin present, Figure 3.

Figure 3 shows there is approximately a three fold increase in tumour levels of doxorubicin were found for the conjugate for all time intervals from 2 min up to 24 hours. After this period, there is no difference between conjugate or the free drug. The elevated levels of the conjugate were at their highest 5 min after injection.

Example 4

In this example the pharmacology of succinolyated dextrin doxorubicin is determined and is presented in Table 2. Twenty four C57 black mice were injected subcutaneously (s.c.) with 10^5 B16F10 murine melanoma cells as described above and then monitored daily for well-being and the presence of palpable tumours. When the tumours were palpable, mice were randomly assigned into groups of six and their tumours measured with a micrometer gauge. Tumour size and mouse body weight is recorded. Each group is then injected intra-peritoneally with either sterile saline (negative control), free doxorubicin (5mg kg^{-1}) in sterile saline or dextrin-doxorubicin (11.8 wt%, 34% succinolyation) at either 5mg kg^{-1} or 10mg kg^{-1}, on days 0,1 and 2. The mice were monitored daily and tumour size and body weight recorded. Once the tumour area exceeded 2.89 cm^2 the mice were culled according to UKCCCR guidelines. Mouse survival is then expressed as % T/C (test/control saline).

The animals treated with doxorubicin (5mg kg-^{1}) displayed a drop in body weight consistent with toxicity. However all mice tolerated the dextrin –doxorubicin conjugate at both doses. The higher dose (10mg kg^{-1}) equates to approximately 2 mg of conjugate. As shown in Table 2, dextrin- doxorubicin conjugate resulted in a T/C of approximately 140% indicating anticancer activity. In contrast, free doxorubicin was not active in this experiment.

Example 5

The tumour model used was B16F10 murine melanoma. Viable tumour cells (10^5) were injected subcutaneously into C57/BL mice near the base of the neck. When

tumours were visible ^{125}I-labelled dextrin (100µl, 5 x 10^5 (cpm) was injected i.v. into the tail vein and the mice were culled at 5 min and 1h. A blood sample was taken and the mouse weighed. The major organs were removed and homogenised in a known volume of DI water. Samples (3 x 1ml) of each tissue were taken and assayed radioactivity. The total amount of radioactivity per organ was expressed as the percentage of the injected dose or as percent of the dose injected per gram of organ.

Figure 4 shows the effect of the degree of dextrin succinoylation on biodistribution of ^{125}I-labelled Dextrin at 34mol% after i.v. administration. Over time it can be noted that there is an decrease in the overall % recovery of the injected dose. Example of organ recoveries, tumour levels increased from 2.5% dose (5 min) to 7.3% dose (1h). Liver levels increased from 10.8% dose (5min) to 11.5% dose (1h) and spleen levels increased from 5.5% dose (5 min) to 9.7% dose (1h). All of the other organs showed a decrease in the % recovery.

Example 6

Figure 5 shows a comparison of the 1 and 34 mol% modified ^{125}I-labelled dextrin at 5 min. At five minutes the overall recovery is greatest in the 34mol%, the tumour % recovery rose from 0.6% dose to 2.5% dose after an increased succinoylation and there was over a two fold difference in the other major organs except the kidney where the % recovery dropped from 15.7% to 11.5% of the injected dose.

Example 7

Figure 6 shows a comparison of the ^{125}I-labelled dextrin at 1h. At 1h the accumulation in the kidneys is greater than at 1mol% modified dextrin the 34mol% giving 7.3% dose. The overall recovery for both mol% modified dextrin has decreased over time.

Example 8

Figures 7 show comparisons of recovery in the i.p. wash in the tumour bearing mice. The dextrin at 34mol% is being retained in the i.p. cavity for longer than the other modified polymers

ART 34 AMDT

1. A polymer drug conjugate comprising:

i) at least one anti-cancer agent; and

ii) a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterised in that the stability of the polymer drug conjugate is enhanced.

2. A polymer drug conjugate according to claim 1, wherein said dextrin is succinoylated to at least 30mol%.

3. A polymer drug conjugate according to Claim 2, wherein said dextrin is succinoylated from 30% to 40mol%.

4 A polymer drug conjugate according to Claim 3, wherein said dextrin is succinoylated from 32% to 36mol%.

5. A polymer drug conjugate according to Claim 4 wherein said dextrin is succinoylated to about 34mol%.

6. A polymer drug conjugate according to any of Claims 1-5 wherein the percentage of α-1-6 linkages in the dextrin is less than 10%.

7. A polymer drug conjugate according to Claim 6 wherein the percentage of α 1-6 linkages in the dextrin is less than 5%.

8. A polymer drug conjugate according to any of Claims 1-7 wherein the molecular weight of the dextrin is in the average molecular weight range 1000-200000.

9. A polymer drug conjugate according to Claim 8 wherein the molecular weight of the dextrin is in the average molecular weight range 2000-55000.

10. A polymer drug conjugate according to any of Claims 1-9 wherein the dextrin

AMENDED SHEET

ART 34 AMDT

contains more than 15% of polymers of DP greater than 12.

11. A polymer drug conjugate according to Claim 10 wherein the dextrin contains more than 50% of polymers of DP greater than 12.

12. A polymer drug conjugate according to any of Claims 1-13, wherein said anti cancer agent is selected from the group consisting of: cyclophosphamide; melphalan; carmusline; methotrexate, 5-fluorouracil; cytarabine; mercaptopurine; anthracyclines; daunorubicin, doxorubicin; epirubicin; vinca alkaloids; vinblastin; vincristine; dactinomycin; mitomycin C; taxol; L-asparaginase; G-CSF; cisplatin; carboplatin

13. A pharmaceutical composition comprising a polymer drug conjugate according to any of Claims 1-12.

14 A pharmaceutical composition according to Claim 13 wherein said composition comprises a diluent, carrier or excipient.

15. The use of a polymer drug conjugate according to any of Claims 1-12 for the manufacture of a medicament for the treatment of cancer.

16. A polymer drug conjugate comprising:

i) at least one biologically active agent; and

ii) a dextrin polymer, wherein said dextrin polymer is modified by succinoylation by at least 20mol% characterised in that the stability of the polymer drug conjugate is enhanced.

17. A polymer conjugate according to Claim 16 wherein said agent is an imaging agent.

18. A polymer conjugate according to Claim 17 wherein the imaging agent is tyrosinamide.

ART 34 AMDT

19. A polymer conjugate according to Claim 16 wherein said agent is a diagnostic agent;

20. A polymer conjugate according to Claim 16 wherein said agent is a targeting agent;

21. A polymer conjugate according to Claim 20 wherein the targeting agent is biotin.

22. A method of treatment of an animal subject the method including the administration to the animal a pharmaceutically effective amount of the polymer drug conjugate according to any of Claims 1-12.

23. A method of treatment according to Claim 22 wherein said animal is human.

AMENDED SHEET

TABLE 2

Compound	Dose mg kg^{-1} (day 0,1,2)	Days survival after treatment (mean ± SD)	T/C (%)	Toxic deaths
Control (saline)	-	4.3± 0.5	100	0/6
doxorubicin	5	4.5 ± 0.5^{ns}	103	0/6
Dextrin-Dox	5	6.2 ±0.8*	142	0/6
Dextrin -Dox	10	6.0 ±1.1**	138	0/6

N= 6 ns = not significant * p = 0.0004 ** p = 0.005

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78355 A2

(51) International Patent Classification[7]: A61K 47/48

(21) International Application Number: PCT/GB00/02216

(22) International Filing Date: 19 June 2000 (19.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9914187.1 18 June 1999 (18.06.1999) GB
9930252.3 22 December 1999 (22.12.1999) GB

(71) Applicant *(for all designated States except US)*: **ML LABORATORIES** [GB/GB]; 17 Hanover Square, London W1R 9AJ (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DUNCAN, Ruth** [GB/GB]; School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX (GB). **HRECZUK-HIRST, Dale** [GB/GB]; School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX (GB). **GERMAN, Lisa** [GB/GB]; School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX (GB).

(74) Agent: **HARRISON GODDARD FOOTE**; Tower House, Merrion Way, Leeds LS2 8PA (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

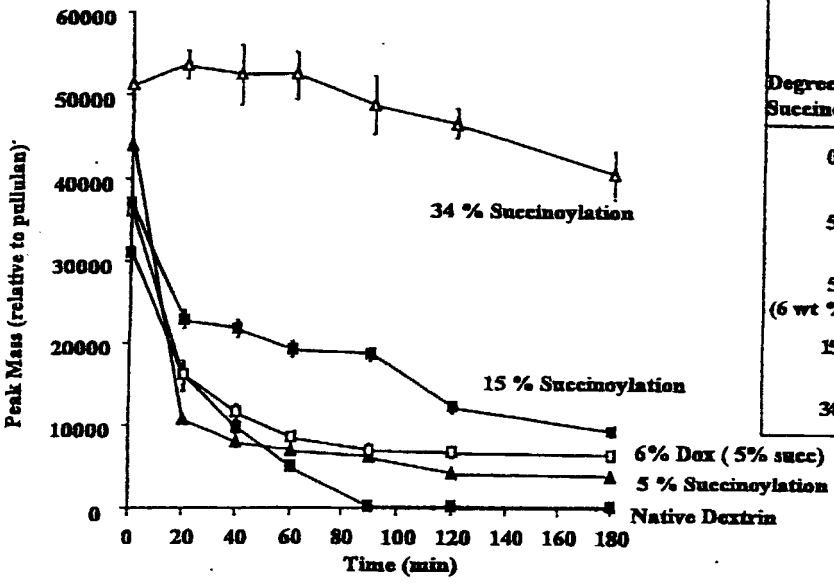
Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/78355 A2

(54) Title: BIOLOGICALLY ACTIVE MATERIALS

(57) Abstract: The invention relates to a polymer drug conjugate for the treatment of cancer comprising a succinoylated dextrin wherein said succinoylation enhances the *in vivo* stability of said conjugate.



n=3 ±S.D.

Degree of Succinoylation	% Reduction of peak mass of primary peak	
	60 min	180 min
0	84	99
5	85	90
5 (6 wt % Dex)	77	92
15	49	75
34	0	20

Figure 1 1/6

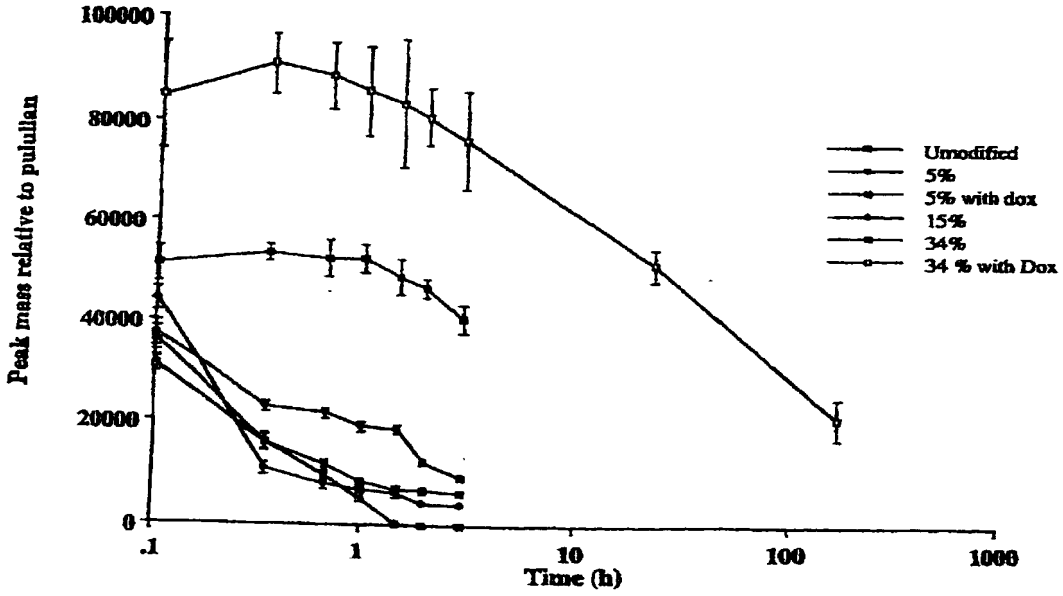

100000
80000
60000
40000
20000
0
Peak mass relative to pulullan
.1
1
10
100
1000
Time (h)
Unmodified
5%
5% with dox
15%
34%
34 % with Dox

Figure 2 2/6

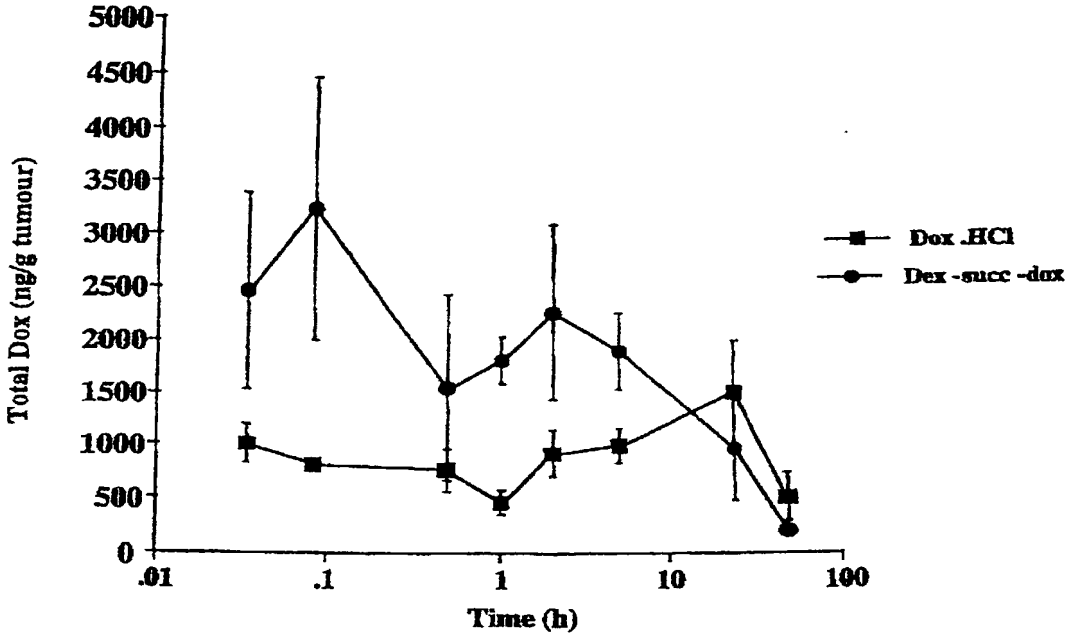

5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Total Dox (ng/g tumour)
.01
.1
1
10
100
Time (h)
Dox .HCl
Dex -succ -dox

Figure 3 3/6

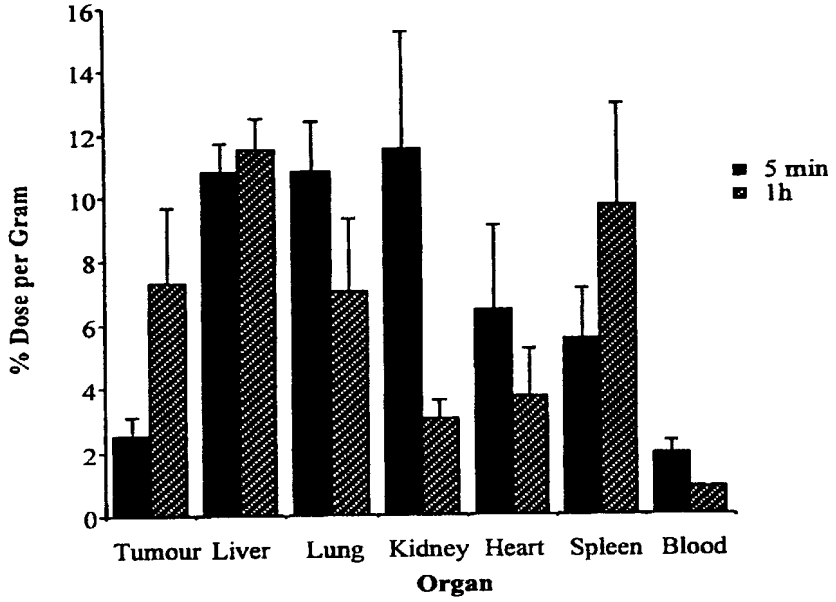


Figure 4

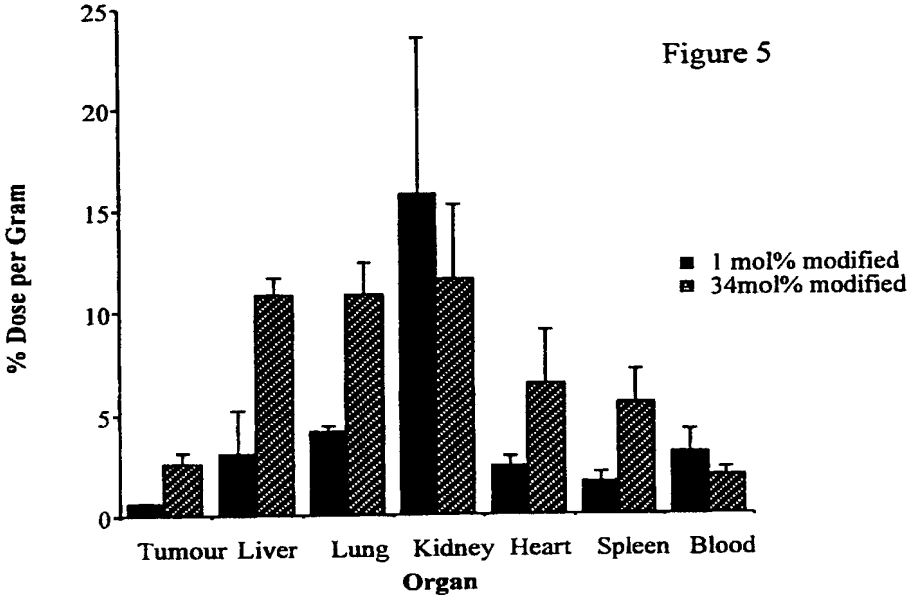


Figure 5

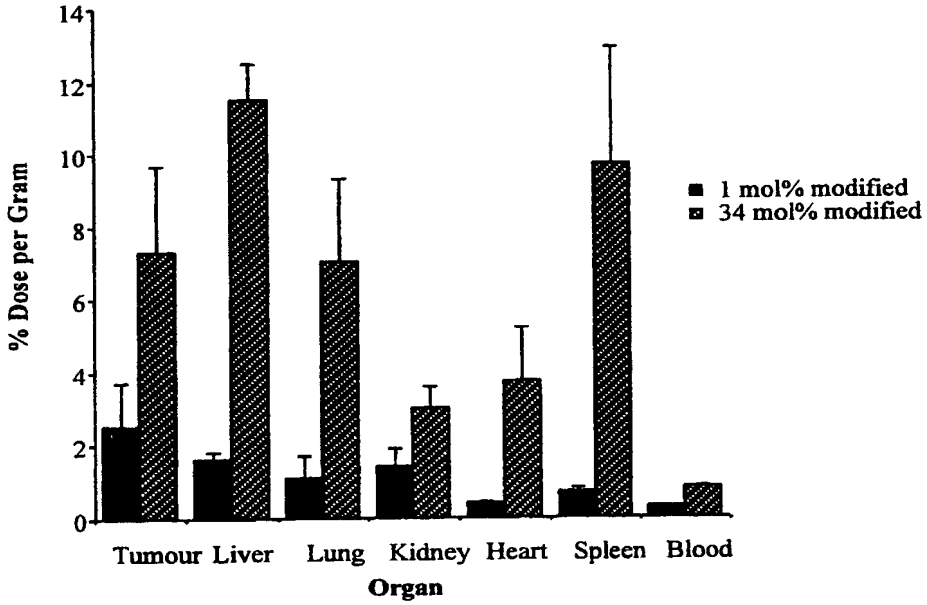


Figure 6

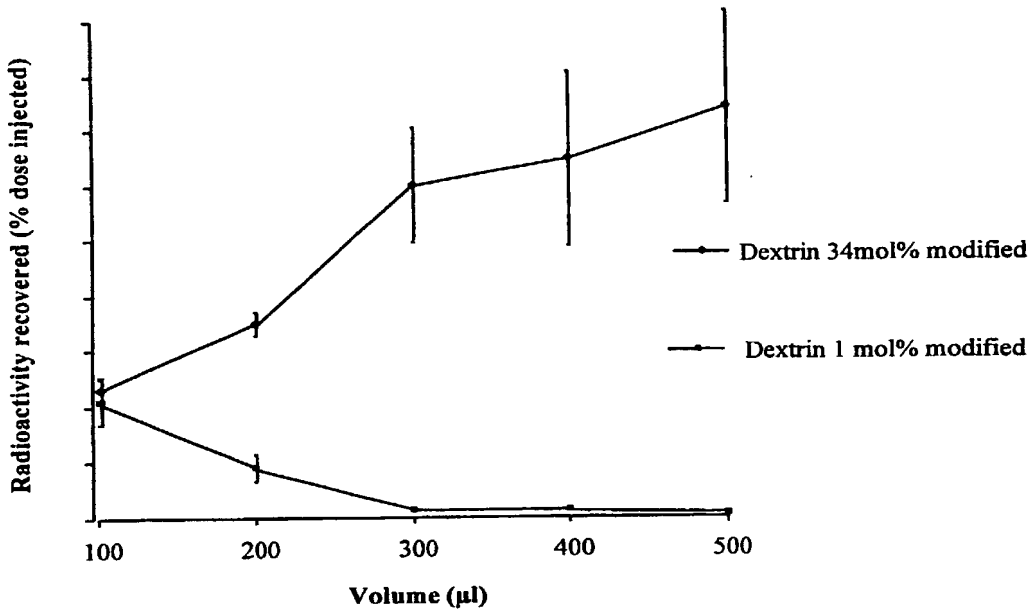

Radioactivity recovered (% dose injected)
100
200
300
400
500
Volume (µl)
Dextrin 34mol% modified
Dextrin 1 mol% modified

Figure 7.

SAS/GLB/:nms 05/02/02 83479

Attorney Ref. No. 5585-61534

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled BIOLOGICALLY ACTIVE MATERIALS, the specification of which

☐ is attached hereto.

☒ was filed on December 17, 2001 as United States Patent Application No. 10/018,608.

☒ was described and claimed in PCT International Application No. PCT/GB00/02216, filed on 19 June 2000, and as amended under PCT Articles 19 on _____ (if applicable).

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses claims and subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 C.F.R. § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the applications(s) on which priority is claimed:

Number	Country	Day/Month/Year Filed	Yes	No
9914187.1	GB	18 June 1999	☒	☐
9930252.3	GB	22 December 1999	☒	

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Number	Filing Date

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT international application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the

SAS/GLB/nms 05/02/02 83479

Attorney Ref. No. 5585-61534

manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/GB00/02216	19 June 2000	Pending
Application Number	Filing Date	Status: patented, pending abandoned

I hereby appoint the practitioners associated with the customer number provided below to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

Customer Number 24197
Klarquist Sparkman

Name	Reg. No.	Name	Reg. No.
BUNKER, Gillian	47,461	ORR, David E.	44,988
BURG, Daniel B.	41,649	PETERSEN, David P.	28,106
CALDWELL, Lisa M.	41,653	POLLEY, Richard J.	28,107
CARLSON, Anne	47,472	RINEHART, Kyle B.	47,027
GIRARD, Michael P.	38,467	RUPERT, Wayne W.	34,420
HAENDLER, Jeffrey B.	43,652	RYBAK, Sheree L.	47,913
HARDING, Tanya M.	42,630	SCOTTI, Robert F.	39,830
JAKUBEK, Joseph T.	34,190	SIEGEL, Susan Alpert	43,121
JONCUS, Stephen J.	44,809	SLATER, Stacey C.	36,011
JONES, Michael D.	41,879	STEPHENS Jr., Donald L.	34,022
KLARQUIST, Kenneth S.	16,445	STUART, John W.	24,540
KLITZKE II, Ramon A.	30,188	VANDENBERG, John D.	31,312
LEIGH, James S.	20,434	WHINSTON, Arthur L.	19,155
MC LEOD, Richard D.	46,921	WIGHT, Stephen A.	37,759
MAURER, Gregory L.	43,781	WINN, Garth A.	33,220
MIRHO, Charles A.	41,199	ZASTROW, Devon J.	50,206
NOONAN, William D.	30,878		

I hereby grant the law firm of Klarquist Sparkman, LLP, the power to insert on this Combined Declaration and Power of Attorney any further identification which may be necessary or desirable in order to comply with the rules of the United States Patent and Trademark Office for submitting this document.

Address all telephone calls to Susan Alpert Siegel, Ph.D. at telephone number (503) 226-7391.

Address all correspondence to the address associated with the customer number provided below:

Customer Number 24197
Klarquist Sparkman

SAS/GLB/mms 05/02/02 83479

Attorney Ref. No. 5585-61534

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of First or Sole Inventor: Ruth Duncan
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom GBN
Citizenship: United Kingdom
Inventor's Signature R Duncan **Date** 16th July 2002

Name of Second Inventor: Dale Hreczuk-Hirst
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom GBN
Citizenship: United Kingdom
Inventor's Signature _____ **Date** _____

Name of Third Inventor: Lisa German
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom GBN
Citizenship: United Kingdom
Inventor's Signature _____ **Date** _____

SAS/GLB/nms 05/2/02 83479 Attorney Ref. No. 5585-61534

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of First or Sole Inventor: Ruth Duncan
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom
Inventor's Signature _____ **Date** _____

Name of Second Inventor: Dale Hreczuk-Hirst
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom
Inventor's Signature _____ **Date** 10/07/02

Name of Third Inventor: Lisa German
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom
Inventor's Signature _____ **Date** _____

SAS/GLB/nms 05/02/02 83479 Attorney Ref. No. 5585-61534

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of First or Sole Inventor: Ruth Duncan
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom

Inventor's Signature _____ **Date** _____

Name of Second Inventor: Dale Hreczuk-Hirst
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom

Inventor's Signature _____ **Date** _____

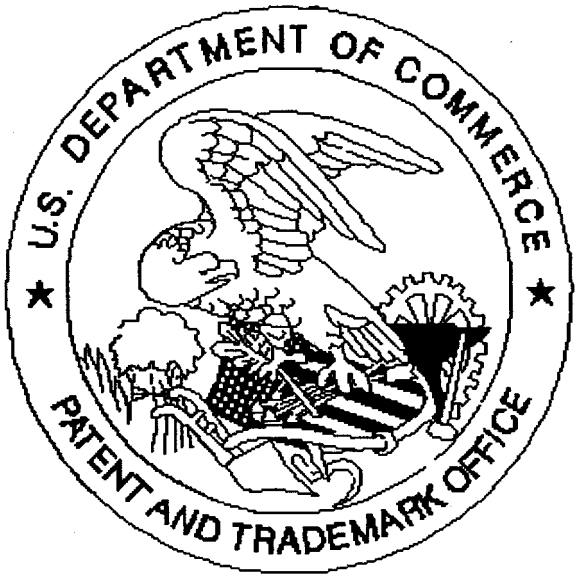
Name of Third Inventor: Lisa Gerrman
Residence: London, United Kingdom
Mailing Address: School of Pharmacy, University of London, 29/39 Brunswick Square, London, WC1N 1AX, United Kingdom
Citizenship: United Kingdom

Inventor's Signature LAGerman **Date** 15/07/02

SCANNED, #18

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ ***Scanned copy is best available.***

Specification pages numbers out of order.